HBsAg Quantification in Clinical Practice

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Several standardized commercial assays for quantification of hepatitis B surface antigen (qHBsAg) are now available. Studies on HBsAg kinetics from Asia and Europe have demonstrated that HBsAg levels are highest during the immune-tolerant phase, become lower during immune-clearance phase and are the lowest in hepatitis B 'e' antigen (HBeAg)-negative inactive low-replicative phase with a rise during HBeAg-negative chronic hepatitis B (CHB). Combined use of hepatitis B virus-deoxyribonucleic acid (HBV-DNA) and HBsAg levels may help in differentiating true inactive carrier state from HBeAg-negative CHB. Several retrospective studies have demonstrated a role for decline in HBsAg level for predicting response and nonresponse to therapy. In HBeAg-positive patients treated with pegylated-interferon (PEG-IFN), a lack of decline of qHBsAg at week 12 predicts nonresponders while a decline of qHBsAg at week 24 predicts responders to PEG-IFN. In HBeAg-negative patients, if at week 12, there is no decline in qHBsAg and the HBV-DNA decline is < 2 log, the patient is unlikely to respond, then stopping of PEG-IFN should be considered. With nucleos(t)ide analogs, the decline in HBsAg is lower than that with PEG-IFN and more marked in patients with HBeAg-positive chronic hepatitis, with elevated alanine aminotransaminase (ALT), thus suggesting that active immune response against HBV is required to lower HBsAg. In patients with HBeAg-negative chronic hepatitis, fall in HBsAg may help in developing stopping rules to reduce the need for lifelong therapy. Information provided by HBsAg is complementary to HBV-DNA and cannot replace the same. Prospective studies on HBsAg kinetics from all regions of the world are required to define optimum time of testing and cutoff levels before stopping rules can be recommended. (J Clin Exp Hepatol 2012;2:75–80)

Hepatitis B virus (HBV) infection is a global health problem affecting >2 billion people worldwide.[1] There are >350 million patients with chronic hepatitis B (CHB), 75% of whom reside in the Asia-Pacific region.[2] Individuals with hepatitis B are at increased risk of developing cirrhosis and hepatocellular carcinoma leading to >1 million deaths each year.[3] Upon entry into the hepatocyte nucleus, the host and viral polymerase repair the partially double-stranded HBV genome to a fully double-stranded covalently closed circular deoxyribonucleic acid (cccDNA), which as a non-integrated minichromosome, acts as a template for transcription of viral genes. Hepatitis B surface antigen (HBsAg) was the first HBV protein to be discovered in 1965 and detection of HBsAg as a qualitative marker continues to be the benchmark for diagnosis of overt HBV infection.[4] Loss of HBsAg with appearance of anti-HBs indicates immunological control of the infection. Despite major advances in treatment of CHB the currently available therapeutic agents do not eradicate infection in most patients. Assay of cccDNA in liver tissue is the most accurate index of infected hepatocytes but the complexity of testing renders it unavailable for routine use. Serum HBsAg has been shown to correlate with transcriptionally active cccDNA.[5] Accordingly, HBsAg clearance with antiviral treatment is considered the closest thing to cure for CHB and confers excellent prognosis in the absence of cirrhosis.[6] While HBV-DNA decline reflects reduction in viral replication, HBsAg decline represents reduction in translation of messenger RNAs produced from transcriptionally active cccDNA.[7] The availability of commercial assays for quantitative HBsAg (qHBsAg) have provided an opportunity to study the kinetics of HBsAg decline during treatment with interferon (IFN) and nucelos(t)ide analogs (NA).

STRUCTURE AND MOLECULAR VIROLOGY OF HEPATITIS B SURFACE ANTIGEN

The open reading frame that encodes for the envelope of HBsAg has three start codons encoding for small (SHBs), middle (MHBs), and large (LHBs) hepatitis B surface

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Abbreviations: ALT: alanine amino transaminase; cccDNA: covalently closed circular deoxyribonucleic acid; CHB: chronic hepatitis B; HBeAg: hepatitis B 'e' antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; NPV: negative-predictive value; PEG-IFN: pegylated-interferon; PPV: positive-predictive value; qHBsAg: quantitative HBsAg; RLU: relative light units
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proteins corresponding to the s, pre-s1+s and pre-s1+pre-s2+s domains, respectively. Following transcription, protein synthesis takes place in the endoplasmic reticulum resulting in 226 amino acid SHB, 281 amino acid MHBs, and 334–345 amino acid LHBs proteins. To form the envelope of a full 42–45 nm virion called *Dane particle*, a well-balanced ratio of HBs proteins is required.[8] The production of surface antigen proteins exceeds the requirement of virion assembly and the surplus proteins are covalently linked by disulfide bonds and secreted as spheres and filaments. Hence, in addition to being the envelope of the infectious HBV particle, HBsAg is also present in the form of defective non-infective empty spheres of 17–25 nm mainly composed of SHBs protein and filaments of 20 nm with variable length composed of SHBs, MHBs, and LHBs. All three forms are detected in the serum of commercial assays as HBsAg with the spherical form being up to 10,000-fold higher than the full infectious virion.[9] These subviral particles may play a role in evading host immune response.[10] There is greater excess of spherical and filament particles in hepatitis B 'e' antigen (HBeAg) viremic individuals as compared to low viremic anti-HBe-positive carriers in whom the decline of filaments parallels that of the virions while a relative excess of spherical particles remains.[11] Some amount of HBsAg may also be produced by HBV-DNA integrated into host genome, an event that occurs in the early stage of HBV infection.[12]

QUANTITATIVE HEPATITIS B SURFACE ANTIGEN ASSAYS

The first assay for qHBsAg using enhanced chemiluminescence was reported nearly 20 years ago but was limited by lack of standardization.[13] Several standardized commercial assay systems are now available. These include Architect HBsAg QT, Abbot Diagnostics; Elecsys II, Roche Diagnostics; ADVIA Centaur HBsAg assay, Bayer; and the Hepanostika HBsAg, Biomerieux. The Architect HBsAg QT is the method most widely used in clinical practice and is a two-step immunoassay with flexible assay protocols called Chemiflex. In the first step the sample and anti-HBs coated with paramagnetic microparticles are combined. After washing, an acridinium-labeled anti-HBs conjugate is added. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture and the resulting chemiluminescence is measured as relative light units (RLUs). The system is fully automated and can detect as low as 0.2 ng/mL of HBsAg. In the Elecsys HBsAg II assay, in the first incubation step the antigen in the sample reacts with two biotinylated monoclonal HBsAg-specific antibodies and a monoclonal/polyclonal sheep HBsAg-specific antibody labeled with a ruthenium complex to form a sandwich complex.[14] In the second step, strepatavidin coated microparticles are added and the complex binds to the solid phase by interaction with biotin and strepatavidin. The results are reported as a cutoff index, and the sample is considered reactive if the index is >1.0.

HEPATITIS B SURFACE ANTIGEN LEVELS DURING DIFFERENT PHASES OF HEPATITIS B VIRUS INFECTION

Like HBV-DNA, HBsAg level varies during the various phases of HBV infection. In 2004, Deguchi et al first reported high qHBsAg in HBeAg-positive as compared to anti-HBe-positive patients and correlation with HBV-DNA level.[15] It has been consistently demonstrated in studies from Asia and Europe that HBsAg levels are highest during the immune-tolerant (IT) phase, become lower during immune-clearance (IC) phase and are the lowest in HBeAg-negative inactive low-replicative (LR) phase with a rise during HBeAg-negative CHB (ENH) phase.[16,17] In a study from Asia, the median HBsAg titers during IT, IC, LR, and ENH were 4.53, 4.03, 2.86, and 3.35 $\log_{10}$ IU/mL, respectively.[16] The median HBsAg titers were similar between genotypes B and C, and serum HBsAg correlated with HBV-DNA only in the IC phase. In the study from Europe the HBsAg levels in IT, IC, LR, and ENH were 4.96, 4.37, 3.09, and 3.87 $\log_{10}$ IU/mL, respectively.[17] There was strong correlation between HBsAg and HBV-DNA during acute hepatitis (R 0.79, $P<0.01$). Association between HBsAg and HBV-DNA was observed in patients infected with genotype D but not with genotype A. Patients with HBV reactivation during follow-up showed >3-fold higher baseline HBsAg with negative-predictive value (NPV) of 95% for HBsAg cutoff of 3500 IU/mL, thus showing promise as a marker for predicting reactivation. The HBsAg/HBV-DNA ratio was significantly higher in LR phase (1.05 Asians and 1.17 Europeans) as compared to all other patients. The discrepancy between HBV-DNA and HBsAg may be due to production of defective particles that outnumber the virions during LR phase. As HBeAg-negative patients who develop reactivation have higher HBsAg and HBV-DNA levels as compared to inactive carriers, several cutoff levels have been proposed.[18–21] With a combination of cutoff values of 2000 IU/mL for HBV-DNA and 1000–2000 IU/mL for HBsAg, inactive carriers can be identified with 94–100% accuracy, thus helping to identify individuals requiring treatment and reducing the need for liver biopsy. However, these observations have to be validated with prospective studies across all genotypes.

PREDICTION OF RESPONSE AND NONRESPONSE TO PEGYLATED-INTERFERON

The role of qHBsAg for monitoring response to treatment of CHB with IFN was first reported for HBeAg-positive CHB in 1994 and for HBeAg-negative CHB in 2007.[22,23] Subsequently, several studies have demonstrated greater HBsAg decline with pegylated-IFN (PEG-IFN) as compared

to NAs, and it may be possible to predict response and nonresponse in these patients.

Hepatitis B 'e' Antigen-positive Chronic Hepatitis B

Baseline HBsAg level of <10,000 IU/mL has been shown to result in higher response to treatment with PEG-IFN.[24] In the study from Hong Kong, 21 of 92 (23%) patients with HBeAg-positive CHB who received PEG-IFN for 32–48 weeks with or without lamivudine (LAM) achieved sustained viral response. The HBsAg cutoff at 300 IU/mL at month 6 could predict sustained viral response (SVR) with sensitivity of 62% and specificity of 89%. Greater than 1 log reduction in HBsAg at month 6 was seen in 9 of 21 (43%) patients with SVR vs 9 of 71 (13%) patients with nonsustained response ($P<0.001$). Combined HBsAg <300 IU/mL and >1 log reduction at month 6 had a sensitivity, specificity, and positive-predictive value (PPV) and NPV of 43%, 96%, 75%, and 85%, respectively, to predict SVR. In a randomized control trial of 221 patients with HBeAg-positive CHB from Europe treated with PEG-IFN-α-2b with or without LAM for 52 weeks, HBsAg decline was compared between responders and nonresponders.[25] Response, defined as HBeAg loss with HBV-DNA <10,000 copies/mL at 26 weeks after treatment, was seen in 43 of 221 (19%) patients. Responders experienced a more pronounced on-treatment decline in qHBsAg at week 52 as compared to nonresponders (3.3 vs 0.7 $\log_{10}$ IU/mL, $P<0.001$). Patients who achieved no decline at week 12 had a 97% probability of nonresponse and no chance of HBsAg loss. Similar results were found in a representative subset of 149 patients followed up for mean of 3 years. The authors recommended that patients who show no fall of qHBsAg at week 12 should be advised to discontinue therapy with PEG-IFN.

Hepatitis B 'e' Antigen-negative Chronic Hepatitis B

In a large multinational study, 127 patients received PEG-IFN-α-2a for 48 weeks, 137 received PEG-IFN+LAM and 122 received LAM monotherapy.[26] The mean HBsAg level was 3.4 $\log_{10}$ IU/mL (range 0.78–4.89). Pre-treatment qHBsAg was higher in patients infected with genotype A as compared to those with genotype D (median 4.11 vs 3.85 $\log_{10}$ IU/mL), but baseline HBsAg could not predict response to therapy. Significant on-treatment decline in HBsAg was noted during treatment with PEG-IFN (–0.71 $\log_{10}$ IU/mL) and PEG-IFN+LAM (–0.67 $\log_{10}$ IU/mL) but not with LAM alone. The HBsAg <100 IU/mL was achieved in 21% patients with PEG-IFN and 17% with PEG-IFN+LAM. End of treatment HBsAg level correlated strongly with SVR and patients with HBsAg of <10 IU/mL had 88% chance of SVR. Thus, qHBsAg at end of treatment could be used as an indicator of anticipated SVR. Notably, of 64 patients with end of treatment HBsAg <380 IU/mL, 16 (25%) had HBsAg clearance at 3 years as compared to none of 134 patients with end of treatment HBsAg >380 IU/mL. The HBsAg clearance at 3 years was achieved by 52% patients with end of treatment HBsAg <10 IU/mL. Both HBsAg ≤10 IU/mL and HBsAg decline >1.1 $\log_{10}$ IU/mL at week 48 were significantly associated with HBsAg clearance at 3 years. Moucari et al treated 48 patients with PEG-IFN-α-2a for 48 weeks, and SVR was achieved in 25% patients.[27] A decrease of 0.5 $\log_{10}$ IU/mL in qHBsAg at week 12 (19% of patients) had a high predictive value of SVR (NPV 90% and PPV 89%). Similarly, a decrease of 1 $\log_{10}$ IU/mL at week 24 (25% of patients) had NPV 97% and PPV 92% for SVR. In another study of 120 patients treated with PEG-IFN, HBsAg decline ≥10% at week 12 was associated with 1 year off-treatment sustained response of 47% and HBsAg clearance of 23% at 5 years.[28]

PREDICTION OF RESPONSE TO NUCLEOS(T)IDE ANALOGS

Reinjnders et al studied 200 patients with CHB treated with entecavir (ETV) or PEG-IFN (HBeAg-positive: PEG-IFN 61, ETV 33; HBeAg-negative: PEG-IFN 69, and ETV 37) and compared the HBsAg kinetics.[29] In the HBeAg-positive population the decline in qHBsAg was higher with PEG-IFN as compared to ETV (mean decline 0.94 vs 0.38 $\log_{10}$ IU/mL; $P=0.07$). Patients who achieved HBeAg loss during treatment with either PEG-IFN or ETV therapy demonstrated similar reduction in HBsAg level. Decline in qHBsAg with ETV was confined to patients with elevated alanine aminotransaminase (ALT) ≥2 ULN and to a large extent to patients achieving HBeAg loss. This finding suggested that active immune response against HBV is required to lower qHBsAg in patients treated with ETV. In the HBeAg-negative population, decline in HBsAg was seen in patients treated with PEG-IFN while there was no decline with ETV (0.56 vs –0.10 $\log_{10}$ IU/mL; $P<0.001$). A study from Korea demonstrated that >1 $\log_{10}$ IU/mL reduction in qHBsAg during treatment with ETV (5 of 28 patients) was associated with higher HBeAg loss (80% vs 30%, $P=0.034$) at 1 year.[30] With tenofovir, HBsAg clearance rates of 3%, 6%, and 8% at 1, 2, and 3 years treatment have been reported with HBeAg-positive patients but none with HBeAg-negative patients.[31] It has been demonstrated that in patients with HBeAg-positive CHB treated for 96 weeks with ETV or LAM and followed up for 24 weeks off-treatment, HBsAg loss was seen in 18/354 (5.1%) with ETV and 10/355 (2.8%) with LAM.[32] Among the 28 patients with HBsAg loss, 96% achieved HBV-DNA <300 copies/mL and 96% achieved HBeAg loss. Similar results have been reported in 162 patients with HBeAg-positive CHB treated with telbivudine (LdT) for 3 years.[33] Treatment with LdT progressively reduced qHBsAg from baseline (3.8±0.6 $\log_{10}$ IU/mL) to week 24 (3.4±0.7 $\log_{10}$ IU/mL), treatment year 1 (3.3±0.8 $\log_{10}$ IU/mL)

and treatment year 3(3.0 ± 1.4 $\log_{10}$ IU/mL; $P<0.0001$). The HBsAg loss was noted in 9 (6%) patients. Three patterns of HBsAg decline were described during the first year of treatment: rapid (≥1 $\log_{10}$ IU/mL) in 32 patients, slow (0–1 $\log_{10}$ IU/mL) in 74 patients and steady levels in 56 patients. Eight of 32 patients with rapid HBsAg decline vs none of 56 patients with steady HBsAg levels achieved HBsAg loss at year 3 ($P=0.0024$). Hepatitis B virus genotype was a significant determinant of HBsAg kinetics with the fastest decline in genotype A patients. In patients with CHB it is easy to start NA, but it is difficult to decide on optimal time for discontinuation. Current guidelines recommend that treatment with NA be discontinued after 1 year of HBeAg seroconversion in patients with HBeAg-positive CHB and at HBsAg seroconversion in patients with HBeAg-negative CHB.[34–36] Despite adhering to these guidelines, the durability of HBeAg seroconversion is not >80% at 2 years off-treatment with LdT.[37] There is some evidence that qHBsAg may help in predicting off-treatment response to NA. A study of 17 patients with 2-year off-treatment sustained response to LdT demonstrated that qHBsAg decline at week 24 and 52 were better predictors of off-treatment response than HBV-DNA decline rates.[38] The HBsAg level <2 $\log_{10}$ IU/mL at week 104 was highly predictive of sustained off-treatment response with PPV 93% and NPV 100%.

PREDICTING RESPONSE IN PATIENTS WITH DUAL INFECTION

Hepatitis B surface antigen quantification has also been shown to be beneficial in predicting response in patients with dual infection with HBV and hepatitis C virus (HCV). Yu et al treated 120 patients with HBeAg-negative CHB also infected with HCV with PEG-IFN α-2a and ribavirin for 48 weeks (genotype 1; $n=74$) or 24 weeks (genotype 2/3; $n=46$).[39] The baseline serum HBsAg level was low in this cohort with dual infection (median 120 IU/mL) and decreased gradually. Low baseline HBsAg was significantly associated with HBsAg clearance (40% for HBsAg ≤20 IU/mL vs 2.2% for >20 IU/mL; $P<0.05$). A decrease of <50% in HBsAg level from baseline to week 12 was associated with reduced likelihood of HBV-DNA reactivation in patients with baseline undetectable serum HBV-DNA (PPV of 89.5%).

INCORPORATING HEPATITIS B SURFACE ANTIGEN QUANTIFICATION INTO CLINICAL MANAGEMENT OF CHRONIC HEPATITIS B

Several recent publications have discussed incorporating HBsAg quantification in clinical practice.[40–43] While it is true that further studies are required to elucidate the levels of HBsAg, and the rate of its decline, HBsAg quantification is likely to change the rules of initiating therapy and the end points of response to therapy for HBV. Hence, how does one individualize the therapy of patients with HBV using HBsAg quantification with the current knowledge on the subject?

Differentiating Immune Tolerance Phase and Immune-clearance Phase

In HBeAg-positive patients, qHBsAg level of >10,000 IU/mL suggests that the patient is likely to be in the IT phase and should make the clinician consider waiting, especially if ALT is normal or borderline.

Differentiating Hepatitis B 'e' Antigen-negative Chronic Hepatitis and Inactive Carriers

Hepatitis B 'e' Antigen-negative patients with CHB have higher qHBsAg and HBV-DNA levels as compared to inactive carriers. While there is still no consensus as to the qHBsAg level cutoff, levels below 1000–2000 IU/mL are considered as low levels. Patients with both HBV-DNA levels ≤2000 IU/mL and qHBsAg <1000 IU/mL have a low probability of having active CHB, especially in genotype D.

Pegylated-interferon Treatment

Because the response to PEG-IFN occurs in 30% of HBeAg-positive and 20% of HBeAg-negative CHB, it is important to identify early the likely nonresponders and consider stopping of therapy. Early stopping rules with high NPVs may not only help in management of CHB but also encourage patients to consider PEG-IFN as a first-line therapy instead of nucleoside analogs, which may be lifelong.[42]

In HBeAg-positive patients treated with PEG-IFN, a lack of decline of qHBsAg at week 12 predicts nonresponders while a decline of qHBsAg at week 24 can predict responders to PEG-IFN.

In HBeAg-negative patients, if at week 12, there is no decline in qHBsAg and the HBV-DNA decline is <2 log, the patient is unlikely to respond and stopping of PEG-IFN should be considered.

Nucleos(t)ide Analog Treatment

Among patients who have been on nucleos(t)ide analogs, and are HBV-DNA negative, low levels of qHBsAg may predict lower risk of relapse on stoppage of treatment. If HBsAg levels were <100 IU/mL, especially in Asians (genotypes B and C), stoppage of therapy may be considered in such patients.

A rapid decline in qHBsAg levels after clearance of HBV-DNA may identify patients who will clear HBV-DNA long-term.

CONCLUSION

Secretion of HBsAg is a dynamic process that varies with the stage of HBV infection, and HBsAg levels are determined by transcription of specific mRNAs and a complex equilibrium between the virus and host immune system.

The availability of standardized assays for qHBsAg have opened yet another exciting window in the field of hepatology. The information provided by qHBsAg is complementary to the more expensive HBV-DNA and is unlikely to replace the latter. The HBsAg levels could help in differentiating HBeAg-inactive state from HBeAg-negative chronic hepatitis. Moreover, the decline in qHBsAg could help in predicting response and nonresponse in treatment with PEG-IFN and NA, thus tailoring therapy to individual needs, much like for chronic hepatitis C. However, there is a need to study qHBsAg in larger trials covering regions beyond Asia and Europe, thus including all genotypes. With the availability of multiple variables to predict response to treatment including HBV genotype, ALT, HBV-DNA, and qHBsAg, mathematical models need to be developed to predict response in a given patient and establish robust stopping rules.

CONFLICTS OF INTEREST

All authors have none to declare.

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